Editing the Sickle Cell Disease Mutation in Human Hematopoietic Stem Cells: Comparison of Endonucleases and Homologous Donor Templates.

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Public Summary:
Studies were performed to identify the best methods to correct the sickle cell disease-causing mutation in blood forrming stem cells using CRISPR.

Scientific Abstract:
Site-specific correction of a point mutation causing a monogenic disease in autologous hematopoietic stem and progenitor cells (HSPCs) can be used as a treatment of inherited disorders of the blood cells. Sickle cell disease (SCD) is an ideal model to investigate the potential use of gene editing to transvert a single point mutation at the beta-globin locus (HBB). We compared the activity of zinc-finger nucleases (ZFNs) and CRISPR/Cas9 for editing, and homologous donor templates delivered as single-stranded oligodeoxynucleotides (ssODNs), adeno-associated virus serotype 6 (AAV6), integrase-deficient lentiviral vectors (IDLVs), and adenovirus 5/35 serotype (Ad5/35) to transvert the base pair responsible for SCD in HBB in primary human CD34+ HSPCs. We found that the ZFNs and Cas9 directed similar frequencies of nuclease activity. In vitro, AAV6 led to the highest frequencies of homology-directed repair (HDR), but levels of base pair transversions were significantly reduced when analyzing cells in vivo in immunodeficient mouse xenografts, with similar frequencies achieved with either AAV6 or ssODNs. AAV6 also caused significant impairment of colony-forming progenitors and human cell engraftment. Gene correction in engrafting hematopoietic stem cells may be limited by the capacity of the cells to mediate HDR, suggesting additional manipulations may be needed for high-efficiency gene correction in HSPCs.